FOLATE RECEPTOR BINDING CONJUGATES OF ANTIFOLATES

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application Ser. No. 61/097,655, filed Sep. 17, 2008, the entirety of the disclosure of which is incorporated herein by reference.

BACKGROUND AND SUMMARY OF THE INVENTION

[0002] Folic acid (FA), or vitamin B9, is an essential nutrient required by all living cells for proper metabolic maintenance of 1-carbon pathways as well as for nucleotide biosynthesis. This ligand displays extremely high affinity (KD~100 pM) for a cell surface-oriented glycoprotein called the folate receptor (FR), which is a glycosylphosphatidyinositol-linked protein that captures its ligands from the extracellular milieu. The folate receptor (FR) is a tumor-associated membrane protein that binds folic acid (FA) and is capable of transporting molecules bound to folic acid inside cells via an endocytosis mechanism. Immediately after binding, the plasma membrane surrounding the FR-ligand complex will invaginate to form an internal vesicle, called an endosome. The pH of the vesicle lumen is somewhat lowered through the action of proton pumps that are co-localized in the endosome membrane, and this acidification presumably mediates a conformational change in the FR protein to release its bound ligand to allow for cytosolic entry. The FR is also a recognized tumor antigen; and because of this, methods to exploit its presence and function have been explored for possible therapeutic value.

[0003] FR-α distribution in normal adult tissue is restricted to the apical membrane surface of some polarized epithelial cells, including lung, choroid plexus and some glandular tissue. Expression is also high in placental trophoblasts and on the luminal surface of proximal tubule kidney epithelial cells, the latter probably being important for the re-absorption of folates from the urine. However, Elevated expression of the FR-α occurs in several cancer types. Non-mucinous ovarian cancer (the majority of ovarian cancers) was the tumor type first to be associated with "over-expression". Several studies confirmed that ~80-90% of these tumors over-express FR-α. Other gynecological cancers (e.g. ~50% of serous uterine tumors) also over-express the receptor. Although the endometrioid histologic subtype may express the receptor less frequently, it is by far the most common form of uterine cancer. Therefore, it is believed herein that a considerable number of uterine cancer patients may benefit from some form of FR-targeted therapy. Other tumors reported to over-express FR-α to varying frequencies include pediatric ependymal brain tumors, breast, colon, renal and lung tumors, and mesothelioma.

[0004] Although it is generally accepted that FA can be conjugated to virtually any molecule to mediate delivery inside FR-positive cells, not all conjugates can be expected to bind to the FR with the same affinity. It is believed herein for example that large drugs that are linked in close proximity to the FA moiety may, perhaps due to steric interactions, alter the ability of FA to enter the binding pocket of the FR. Further, the nature of the drug may also be important, because intramolecular association with the FA might yield a poorly-binding conjugate that may not properly orient itself into the FR. In any case, after FA has delivered the molecule to the target site, it is no longer used. Therefore, it was recognized herein that targeting with a different molecule that had the potential to have a second function in treating a disease state would be useful.

[0005] It has been discovered herein that antifolates are also capable of targeting FR, and in conjunction with releasable linkers are also capable of targeted delivery of molecules to cells that express the FR. However, it has been reported that the relative affinity of such antifolates is widely varying, as determined at 4° C., which has hindered the use of antifolates as targeting ligands. It has also been unexpectedly discovered herein that the relative affinity of antifolates at the folate receptor, as compared to folic acid, is temperature dependent. Moreover, it has been discovered that the relative affinity of some antifolates increases with increasing temperature, while the relative affinity of other antifolates decreases with increasing temperature. Further, the relative affinity of still other antifolates is relatively invariant with increasing temperature.

[0006] Described herein are compounds, compositions, and methods that include conjugates comprising a folate receptor binding antifolate, at least one releasable linker, and one or more drugs, where the antifolate has a high relative affinity for the folate receptor, as compared to folic acid, at temperatures above 4° C., such as at temperatures above 20° C., at temperatures above 25° C., at temperatures above 30° C., and/or at temperatures that are physiologically relevant, such as physiological temperatures in mammals. Also described herein are compounds, compositions, and methods that include conjugates comprising a folate receptor binding antifolate, at least one releasable linker, and one or more drugs, where the conjugate has a high relative affinity for the folate receptor, as compared to folic acid, at temperatures above 4° C., such as at temperatures above 20° C., at temperatures above 25° C., at temperatures above 30° C., and/or at temperatures that are physiologically relevant, such as physiological temperatures in mammals. In general, the conjugates described herein ore covalent conjugates; however, it is to be understood that the drugs forming part of the conjugates described herein may include other bond forms, including but not limited to complexes, such as metal chelates, and the like.

[0007] In one embodiment, compounds, compositions, and methods are described herein that include a conjugate comprising an antifolate having a relative affinity for the folate receptor, as compared to folic acid, of at least about 0.1, at least about 0.2, at least about 0.25, or at least about 0.5, at one or more of the temperatures described herein. In another embodiment, compounds, compositions, and methods are described herein that include a conjugate having a relative affinity for the folate receptor, as compared to folic acid, of at least about 0.05, at least about 0.1, at least about 0.2, at least about 0.25, or at least about 0.5, at one or more of the temperatures described herein.

[0008] In another embodiment, a method for evaluating the folate receptor binding ligand affinity for the FR is described herein. In one aspect, the binding affinity is relative and is compared with folic acid. It is to be understood that the assays described herein may be used to evaluate the relative selectivity and/or specificity of the binding to the folate receptor by competing the folate receptor binding ligand and/or conjugate with folic acid. It